Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Faculty of Natural Resources and Agricultural Sciences

Assessment of changes in algal biomass accrual and leaf decomposition along a gradient of increasing agricultural disturbance

Maisam Ali

Department of Aquatic Sciences and Assessment.
Master´s thesis • 30 ECTS • Second cycle, A2E
Uppsala Sweden 2015

Assessment of changes in algal biomass accrual and leaf decomposition along a gradient of increasing agricultural disturbance

Maisam Ali

Supervisor: Brendan McKie, Swedish University of Agricultural Sciences, Department of Aquatic Sciences and Assessment

Examiner: Maria Kahlert, Swedish University of Agricultural Sciences, Department of Aquatic Sciences and Assessment, Section for Ecology and Biodiversity

Credits: 30 ECTS
Level: Second cycle, A2E
Course title: Independent Project/Degree Project in Biology - Master's Thesis
Course code: EX0565
Programme: Forest as Natural Resource - Master's Programme

Place of publication: Uppsala, Sweden
Year of publication: 2015
Cover picture: Copyrightexpired.com
Online publication: http://stud.epsilon.slu.se

Keywords: Leaf decomposition, Algal biomass accrual, Stream biodiversity

Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Faculty of Natural Resources and Agricultural Sciences
Department of Aquatic Sciences and Assessment

Contents

Summary

1. Anthropogenic activities may impact stream ecosystems by distorting the energetic linkages between the aquatic and adjacent terrestrial habitats, or by modifying the aquatic environment directly. These impacts can affect biodiversity, ecosystem functioning, and stability in the stream ecosystem, all of which may compromise ecosystem services of importance to humans. Bio-monitoring schemes are required to assess such impacts.
2. Traditional biomonitoring focuses on the diversity and composition of biological groups, but there is increasing interest in monitoring ecosystem processes. I quantified two key ecosystem processes (litter decomposition and algal biomass accrual) to study the effect of different land use on stream ecosystem functioning. Ten streams were studied, representing a gradient of increasing agricultural land use in Östergötland County, Sweden, ranging from forested reference streams to heavily impacted agricultural sites.
3. Litter decomposition was assessed using the litter bag method, with coarse bags allowing access to invertebrates and fine mesh bags blocking invertebrate access. Thus decomposition rates in the fine mesh bags reflect microbial activity, while the coarse bags reflect microbial and detritivore activity combined. Algal biomass accrual rates was assessed by exposing tiles in the stream for a month, after which time the tiles were retrieved and algal biomass assessed.
4. Litter decomposition increased asymptotically along the land use gradient. This increase was associated with increased nutrient (N, P) flows from the fields to the streams. This increase was stronger in the coarse mesh bags than in the fine mesh bags, indicating stronger invertebrate than microbial responses to the gradient.
5. Little difference in algal biomass accrual was observed along the land use gradient despite the fact that there was an increase in nutrients, possibly reflecting elevated sediment loads to the most agricultural streams, which results in a poor light environment for algal growth.
6. My results showed that not a single assay is enough to give a complete picture of stream health, highlighting the value of multiple functional assays as a complement to traditional taxonomy-based monitoring, which can capture potentially contrasting impacts of human disturbances on different ecosystem processes.

Introduction

Streams and river (i.e. lotic) ecosystems, characterized by unidirectional flow from headwaters to the sea, are integral to nutrient cycling in both natural and anthropogenically modified landscapes, and deliver fundamental ecosystem services, including fresh drinking water and recreational services such as fishing and bathing. Although streams and rivers often have substantial capacity to buffer disturbances, the range of multiple stressors streams are subjected too frequently reduce ecological integrity (Matthaei *et al.,* 2010; Woodcock & Huryn, 2005). This may be reflected in losses in the diversity of running water life (microbial life, macro invertebrates, large animals, algae etc) and changes in ecosystem physiochemistry (Allan, 2004; Boon, 2000; Benke 1990; Dynesius & Nilsson 1994; Lenat, 1984). Streams that are facing declining ecosystem integrity are typically characterized by declining water quality, increasing algal bloom production, loss of aquatic diversity and a decline in volume of water etc. (Lenat, 1984).

A major driver of environmental degradation worldwide is agricultural land management. Since the 1960`s the global population of humans has almost doubled, driving a dramatic increase in food production, which in turn has entailed an intensification of agricultural land-use practices (for example pesticide and fertilizer use). Works by Alexandvatos & Bruinsma (2012) predicted that by 2050 more land will be converted to agricultural land in developing countries (Bremner *et al.,* 2010; Godfray *et al.,* 2010; Gibbs *et al.,* 2009). Conversion of forests into agricultural land can have marked influences on aquatic environments (Arheimer & Liden, 2000; Lenat, 1984). This is because of the tight linkages between aquatic ecosystems, the surrounding landscape and terrestrial life. For example, agricultural fields are susceptible to increased runoff following rain. The runoff washes away soil particles and fertilizers, and delivers them to streams draining the landscape. Excess nutrients entering streams can shift the functioning of stream food webs (Woodward *et al.*, 2012), while excessive sediment inputs may cover important habitats in the streams, hence excluding some life forms from their preferred habitat (Zweig & Rabeni 2001).

The impacts of agriculture on stream ecosystems thus need to be carefully monitored, using both novel and existing techniques. Quantification of ecosystem processes is one novel approach that can give insight into how ecosystems actually work, as opposed to what they are made up of (Gessner & Chauvet, 2002). The responses of two approaches for assessing ecosystem functioning, a leaf-decomposition assay (Gessner & Chauvet, 2002) and an algal biomass accrual assay (Hladyz *et al.,* 2011), to an agricultural nutrient gradient is a major focus of this Master`s thesis. In this introduction I begin by briefly introducing the topic of stream water chemistry, and then present the topic of energy flow in stream ecosystems. I then provide an overview of methods for assessing impacts on ecosystem integrity, including functional approaches, before introducing my study, and the approach taken to address impacts of agriculture on water chemistry and ecosystem functioning.

Water chemistry:

Streams are characterized by a range of physiochemical parameters, e. g. nutrient status, pH, alkalinity, suspended sediments, and hydrological profile and so on (Asonye *et al.,* 2007; Giller & Malmqvist, 1998). These parameters should vary within certain bounds in order to maintain the integrity of the stream ecosystem, particularly because different life forms in the stream survive and function best within certain physiochemical ranges. These physiochemical properties of the stream are regulated by a number of variables that are specific to each stream catchment and to its tributary subcatchments including catchment geology, soil characteristics, and characteristics of riparian vegetation. Additionally, anthropogenic factors including land use activities in the catchment, particularly agriculture, urbanization and forestry activities, can have a strong influence on stream physico-chemical attributes, biodiversity and ecosystem functioning (Giller & Malmqvist, 1998, Woodward *et al*., 2012).

Energy flow in lotic systems:

The concept of an ecological food web describes trophic interactions and energy flows between different life forms within the ecosystem (Pace *et al*., 1999; Winder & Schindler, 2004).The trophic web typically comprises the primary producers, which are photosynthesizing organisms, and consumers, including (i) herbivores that feed on the green plants, (ii) decomposers, for example microbes and detritivores that degrade dead organic matter (especially leaf litter), and (iii) predators that prey on the detritivores and herbivores (Cummins *et al*., 1973). Also, in the stream ecosystem, different trophic levels interact to maintain the food chain that exists within them. Balanced trophic interactions within the stream ecosystem generally favor efficient nutrient cycling with in the trophic levels (Carpenter *et al*., 1985; Pace *et al.,* 1999).

However, today truly pristine ecosystems are hard to find in many regions of the world, as the chemistry of most streams and their life forms have strongly being impacted by anthropogenic activities including intensive agriculture (Karr, 1991; Malmqvist & Rundlle, 2002). In one study, (Lenat,1993) found that streams draining intensively managed agricultural fields saw a declining species community richness of groups like Trichoptera, Ephemeroptera and Plecoptera, known to be intolerant to pollution. The shift from less tolerant taxa to more tolerant taxa in the agricultural streams were due in part to runoff from the agricultural sites, especially additions of nutrients and particular organic matter. This illustrates how water quality changes can impact diversity and composition of species (Gannon & Stemberger, 1978; Meyer *et al.,* 1988; Rosenberg *et al.,* 2004).

Biological assays to measure aquatic ecosystem integrity:

Ecological integrity can be evaluated by comparing the composition and dynamics of an ecosystem, and comparing how these vary along a gradient of anthropogenic stresses ranging from strongly stressed to pristine (Karr, 1991). Up to now, most research on water management has evaluated ecosystem integrity based on assessments of the diversity and structure of biological assemblages. Studies on ecosystem integrity mostly use fish, macroinvertebrate assemblages, (Norris *et al.,* 2000) benthic algal composition and/or macrophytes (Norris & Thoms, 1999) as indicator groups to evaluate the structural integrity of streams. This approach is now widespread and provides a useful framework for evaluating integrity. However, it also has some limitations. In particular, ecosystem integrity embodies two inter-related components (functional and structural integrity) (Cummins, 1974) The structural component of an ecosystem can be described by the qualitative and the quantitative changes in the biological diversity patterns and their corresponding resources at a unique time and place (Vitousek *et al.,* 1997), whereas the functional component of ecosystem integrity relates to how an ecosystem uses and transforms nutrients and energy (Bunn & Davies, 2000; Gessner *et al.,* 1999). This is reflected in rates of important ecosystem level processes i.e. specific biological activities that run with respect to each biological diversity pattern exhibited at a specific time and place. Thus, in order to make comprehensive ecosystem evaluations, an evaluation of the functional integrity of the ecosystem may often be needed to complement the structural integrity evaluations. Bunn & Davies (2000) suggest that the structural and functional attributes examines separate aspects of the same system, therefore both aspects should be considered to get a comprehensive assessment of the ecosystem integrity. However, while it is well recognized that anthropogenic stress can impact ecosystem functioning, it is still often not clear how to assess how changes in functioning and ecosystem integrity are related.

Assays for measuring ecosystem functioning:

There are a variety of functional assays (Palmer & Febria, 2012) that may be used in stream bioassessment. Examples of some of the functional assays include the evaluation of benthic algal biomass accrual, measurement of respiration rates by microorganisms in a community, determination of respiration rate from sediments, determination of secondary growth of macroinvertebrates, and measurements of N_2 uptake rates in biofilms (Bunn *et al.,* 1999). The most appropriate assay to use depends on the stress type imposed on the stream. Thus there is need to know the nature of the stress before choosing a particular functional assay, which remains challenging due to the lack of baseline information on responses of different functional metrics to different disturbances. For example, when evaluating how alterations in light patterns/intensity affect streams ecosystems functioning, a good functional assay to use is likely to be the primary production of the benthic algal community (Hladyz *et al.,* 2011). Changes in canopy cover of the riparian vegetation (Sabater *et al.,* 1998) and increases in turbidity of the stream (Bunn & Davies, 2000) associated by intensified land use are factors that alter light reaching the streams, and algal biomass accrual is likely to be a good assay for tracking effects of these disturbances.

The productivity of algae and other primary producers is a basal process in the functioning of stream food webs, with algae in particular providing an important, high quality food for invertebrates and other consumers (Lamberti *et al.,* 1989). Algal biomass accrual – the sum total of changes in algal standing stocks influenced by algal productivity and grazing pressure and other distrurbances – is particularly sensitive to agricultural disturbance (Jarvey., *et al.,* 2006; Sharpley & Withers, 1994). Two of the most important large scale impacts on streams associated with agricultural land use are the runoff of nutrients and sediments increased runoff of phosphorous and nitrogen to streams stimulates algal growth which may cause a buildup of algae greatly exceeding the resource demands of consumers. Algae not consumed by herbivores will decay. The decaying process consumes large amount of oxygen in water. Insufficient oxygen in the water reduce several of, plants and animals (Kohler & Soluk, 1997). Apart from nutrients, and additional problem in agricultural landscapes is an increased runoff of sediments like clay and silt arising from erosion, which can increase the water turbidity. If the concentrations of the particulate matter are high enough, they may alter the light reaching the water. This may result to a declining photosynthetic rate by the plants in the stream (Grantz *et al.,* 2003; Yentsch, 1962).

Another important functional bioassay technique is the leaf decomposition assay. It is well documented that leaf litter is a major source of energy in streams, especially well shaded headwaters (Canhoto & Graca, 2006), where leaf litter from the riparian zone constitutes a major source of organic matter. The decomposition of leaf litter reflects the activity of microbes and detritivore invertebrates that colonize the fallen plant litter, incorporating the energy and nutrients contained in the litter into biomass (i.e. fungal and invertebrate biomass), or break the litter down to smaller particle sizes (Wallace, Webster & Cuffney, 1982). Fungal and invertebrate biomass in turn can be consumed by other trophic levels, while fecal particles are consumed by filter and deposit-feeding invertebrates (Cummins & Klug, 1979). Leaf decomposition is underpinned by microbial enzymatic catalysis, where enzyme activity speeds are determined by temperature and water chemistry (Tonin & Hepp, 2011; Alster *et al.,* 2013). Further factors that influence litter decomposition include the species identity and quality of the litter itself, species differ in concentrations of nutrients and refractory compounds including lignin (Moorhead *et al.,* 1999), which in turn affects the biomass, abundance and species composition of leaf eating detritivores. These detritivores, often called shredders, are responsible for the bulk of leaf mass loss (Hieber & Gessner, 2002).

My study: approach and predictions:

I studied both algal biomass accrual and leaf decomposition in ten streams and they ranged from pristine sites flowing through forests, to sites impacted by both nutrients and sediment in farmlands. I tested the following hypotheses: (i) agricultural streams will be generally characterized by higher nutrient loadings, turbidity and sediments compared to the forest sites; (ii) increasing nutrients will drive increases in both decomposition and algal biomass accrual, due to bottom-up stimulation of algae and microbes, unless sediments hinder the activities of these organisms; (iii) detritivore abundances will decline with increasing nutrient loadings, reflecting harsher conditions in the more agricultural sites.

Material and Methods

During autumn 2012, ecosystem functioning was assessed in ten streams representing a land use gradient from fully forested to predominantly agricultural catchments. The study sites were located in the province of Östergötland in the south of Sweden (Fig 1, Table 1). The forest type was composed of mixed deciduous and coniferous vegetation (*Betula Pendula, Fraxinus excelsior and Picea abies*). All streams had intact riparian vegetation, ranging from 5-10m width for the agricultural streams, to extensive forest around the non-agricultural sites. All the streams had a rocky bottom (with predominantly, cobbles and some gravel) and some of the streams were rich in nutrients.

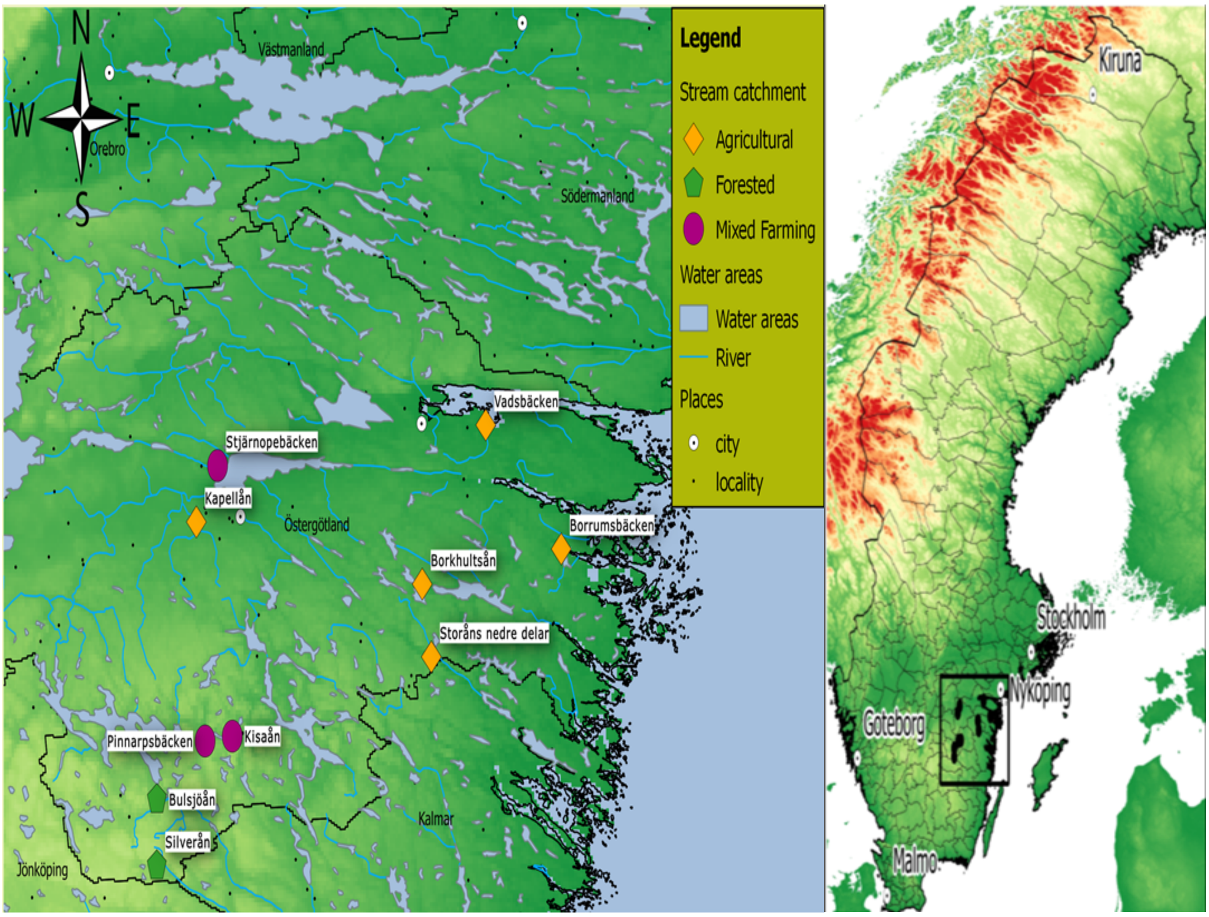

Fig. 1: GIS Maps displaying site locations of the ten different streams types (Agricultural, Forested and Mixed landuse) in south of Sweden.

Table 1: Selected physico-chemical features of the investigated streams.

Pair	Stream name	Nature of stream catchment	Location	Slope (%)	pH	Turbidity	NO_2+NO_3-N(µg/l)	NH_4N(µg/l)	Tot._P(µg/l)	PO_4-P(µg/l)
1.	**Kisaån**	**Mixed**	57°58′N15°35′E	1.74	7.5	0.000	4	9	8	2
2.	**Stjärnopebäcken**	**Mixed**	58°30′N15°33′E	3.49	7.7	12.940	322	26	107	46
3.	**Pinnarpsbäcken**	**Mixed**	57°58′N15°30′E	2.61	7.6	93.391	255	4	13	3
4.	**Bulsjöån**	**Forested**	57°51′N15°21′E	1.3	7.1	0.000	68	41	12	3
5.	**Silverån**	**Forested**	57°43′N15°21′E	2.1	7.2	0.000	39	5	11	4
6.	**Storåns nedre delar**	**Agricultural**	58°8′ N16°13′E	1.74	7.5	0.200	156	34	41	15
7.	**Borkhultsån**	**Agricultural**	58°16′N16°11′E′	0.87	7.6	0.000	13	6	11	2
8.	**Borrumsbäcken**	**Agricultural**	58°20′N16°37′E	3.05	7.4	18.467	1242	78	173	112
9.	**Kapellån**	**Agricultural**	58°23′N15°29′E	2.18	7.7	126,992	1580	78	181	136
10.	**Vadsbäcken**	**Agricultural**	58°35′N16°23′E	0.87	7.8	102.517	1269	147	358	225

Algal biomass accrual assessment:

Algal biomass accrual was quantified using the "Tile Method" (Lamberti & Resh, 2006; Sabeter *et al*., 1998). Unglazed bathroom tiles were installed in the streams and left for colonization by algae. At each study site, eight tiles were installed during August, in four groups of two. Each group of two tiles were tied to a metallic frame and fixed to the stream bed with the aid of two metallic bars. The tiles remained in the water for 40 days during the months of August and September and the Bentho Torch was then used to collect-measure the algal biomass accrual on tiles. The Bentho Torch is an instrument that allows quick and easy measurement of algal biomass (as chlorophyll) from stream substrates, based on fluorescence of cholorophyll (Kahlert and McKie, 2014). Final algal biomass was quantified as µg chlorophyll a per square centimeter of tile area, representing the total growth of algae per square centimeter during the study period.

Fig.2: Tiles installation and bentho torch measurements. Photos: Amélie Truchy

Leaf decomposition trial

I studied decomposition of birch (*Betula pendulum*) leaf litter, collected from mature birch trees just upon abscission (when the leaves were yellow and falling down) and air dried in the lab. The air dried samples were weighed and put in labeled mesh bags in standard weights of 5±0.1 g)(M1, i. e. initial mass of each bag, at time zero) for coarse and fine bags (sizes 1 cm

vs 500 mm) respectively. For each site, 5 replicates were prepared for both the coarse and the fine bags. The purpose of the fine bags (0.5mm) was to prevent leaf shredding macroinvertebrates from entering the bags while still allowing microbial colonization, whereas the coarse mesh bags (10mm) were designed purposely to give access to shredders that colonize the leaves and readily can decompose them.
The replicate bags were then taken to the study sites for installation in September 2012. At each site, five iron bars staked out at five evenly spaced points in the middle of the stream over a reach of approximately 20m. On each bar one fine and coarse bag were tethered. Temperature loggers were attached to one bar in every site and set to record temperature hourly.
The bags were recovered after 40 days from the first day of installation, during November. In the lab each labeled sample bag was open and poured in a tray of water to remove debris and other unwanted matter (rocks, sand, twigs). Invertebrates were separated from the litter and were preserved in 95% ethanol, with individuals classified as leaf eating detritivores (also called "shredders") counted. After removing unwanted matter, the leaves were removed from the water and put in an open box and dried in an oven at 50 °C. After drying for 48 hours the leaves were weighed. The measured weights were then recorded as weight after decomposition of leaf litter (M2, mass remaining i.e. Mass of mesh bag after a particular time interval). Each litter sample was further ground, weighed and then ignited at 550 degree Celsius for 4 hours. The remaining ash was then weighed, in order to estimate mineral ash and this value was used to calculate ash free dry mass (AFDM) (Swift et al 1979).

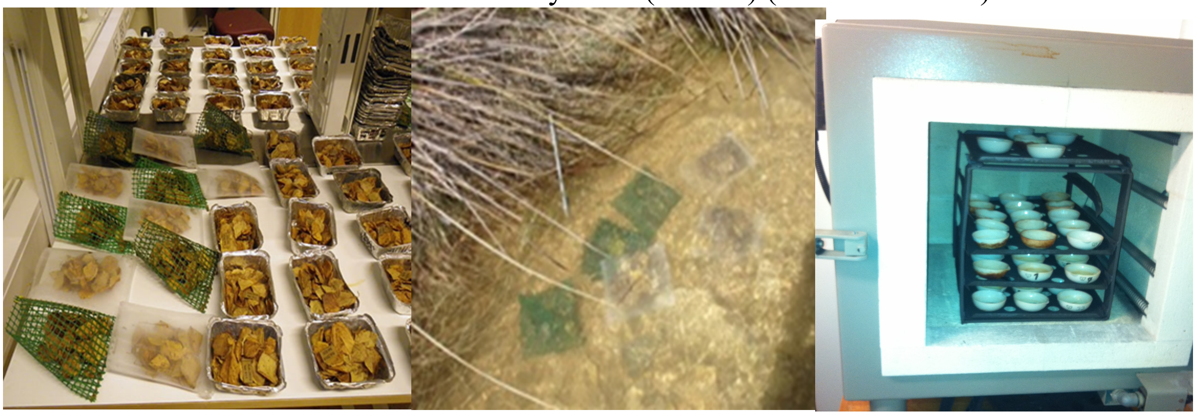

Fig. 3: Collection, installation and oven drying of leaf litter samples.

Decomposition rate was calculated using the following negative exponential equation which has been shown to best represent the non-linear trajectory of decomposition (Benfield, 1996):

$$-k = \frac{\ln\left(\text{mass}_{\text{remaining}} / \text{mass}_{\text{initial}}\right)}{\text{Time (days)}}$$

Statistical analysis:

My sites represented gradients of increasing input, and could not easily be divided into distinct categories. Hence I relied on regression and multivariate statistics rather than ANOVA for analyzing my data.
To address hypothesis (i) I conducted a principal component analysis (PCA) of the streams physicochemical characteristics using Minitab (version 16), with a focus on identifying sites characterized by high sediment and nutrient loadings. The data included were turbidity, silicate, conductivity, alkalinity, sulphate, pH, ammonium, k-fine degree day, k-coarse degree and total algae concentration. Following the PCA analysis, I further assessed how the main environment variables changed along the nutrient gradient using regression analysis. Total P was chosen as the representative measure of nutrients, but was strongly correlated with other

nutrient measures (e.g. TIN, PO_4). The regressions between total P and the other environmental parameters are intended to identify strongly associated variables, but do not imply causality (i.e. a relationship between Total P and turbidity should not be interpreted as meaning Total P drives turbidity).
To assess hypothesis (ii) I first tested the response of leaf decomposition and final algal biomass to the nutrients using linear regression. I chose Total P as the predictor variable best representing the increase in nutrients along the gradient, through use of other nutrient measures (TIN, PO_4) also gave similar results.
However, nutrients were not the only environmental parameter changing along the gradient. Hence, I further used partial least square regression (PLS) to assess the relative importance of the main factors varying along the gradient for explaining variation in decomposition, and in as no significant relationship between the nutrient gradient and decomposition in the fine bags was detected (see Results). PLS identifies both the most important predictor variables for explaining a response variable (via so-called VIP values – Variable Importance on the Projection), but also estimates the regression slope for each predictor variable (Eriksson *et al.*, 1999). All PLS regressions were performed in JMP (SIMCA-P version 10·0). MINITAB software (version 16)
Hypothesis (iii) was addressed by fitting detritivore abundances against Total P in a regression model.

Results

Variation in physico-chemistry among sites:

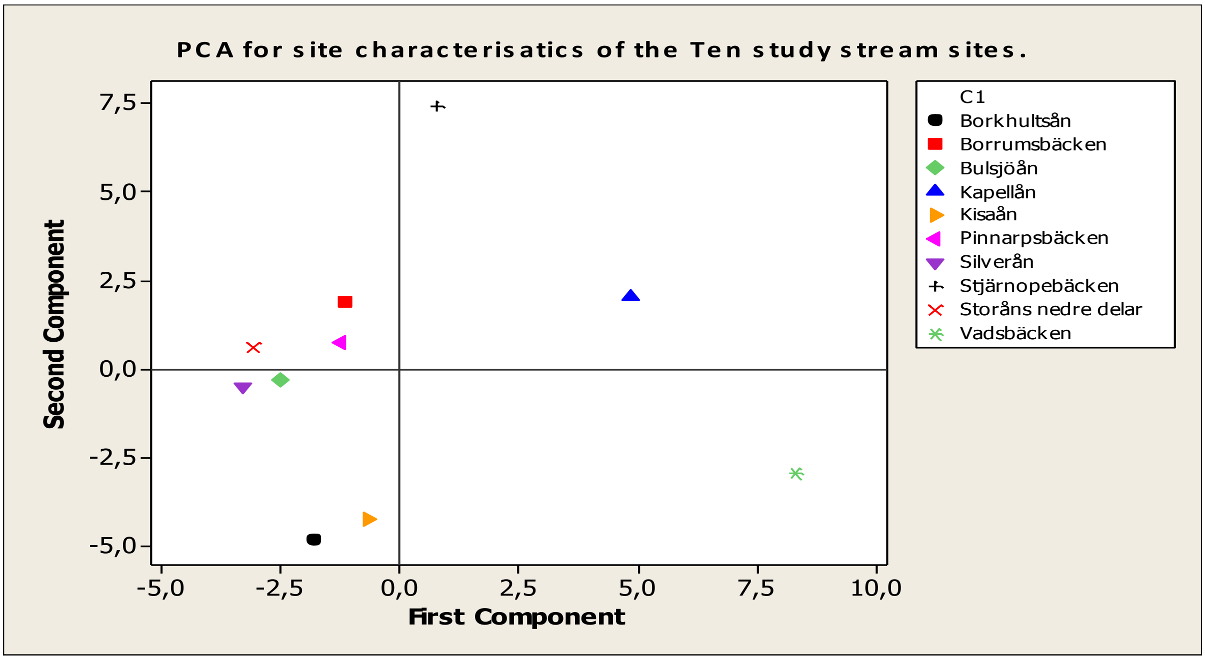

Fig. 4: Principal component analysis (PCA) of site characteristics of ten different catchment streams types in Östergötland County, Sweden.

In a multivariate ordination of the streams abiotic characteristics (Fig. 4), the first axis broadly captured the nutrient gradient, ranging from the more enriched sites Vadsbäcken, Kapellån and Stjärnorpebäcken at one end, with Silverån and Bulsjöån at the other. (i.e corresponding to the “agricultural and forested” land use categories in Fig. 8) The agricultural sites were also more heavily affected by elevated turbidity (Table.1).

Leaf decomposition and algal biomass accrual:

Litter decomposition in both coarse and fine mesh bags both appeared to increase non-linearly along the total Phosphorus gradient (Fig. 5 and 6), but the relationship was only significant in the coarse mesh bags (Fig. 6). There was no significant relationship between final algal biomass and the gradient in total Phosphorus (Fig. 7).

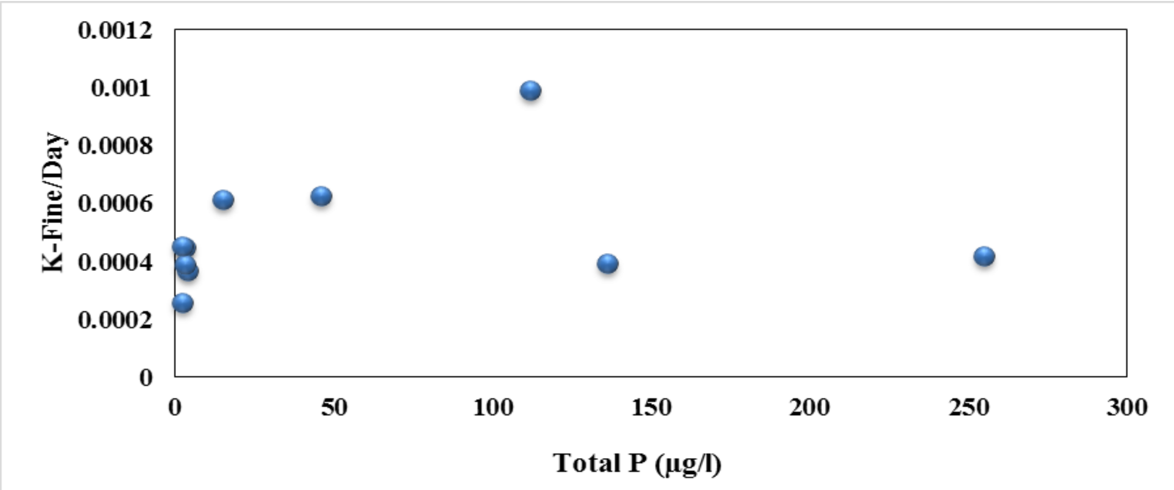

Fig. 5: Response of stream litter decomposition (K-Fine degree day) to stream total Phosphate levels. Litter decomposition in fine mesh bags was positively associated with the streams phosphate levels, but the relationship was not statistically significant. (R^2=0.22, P=0.167).

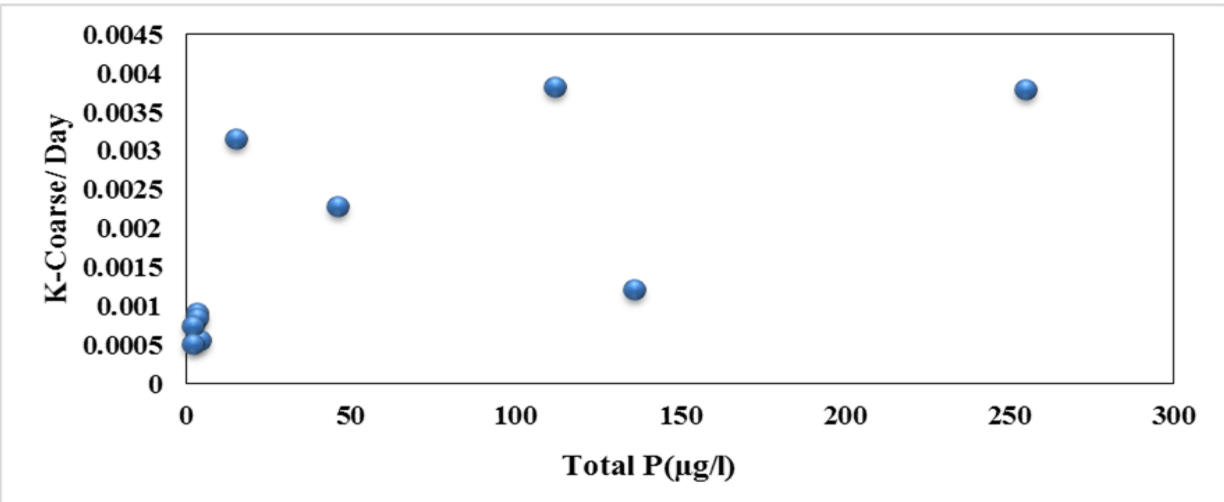

Fig. 6: Response of stream litter decomposition (in k-Coarse degree day) to stream total Phosphate concentrations. Leaf decomposition in the coarse bags is positively associated with the streams total phosphate concentration (R2= 0.6 and P< 0.008).

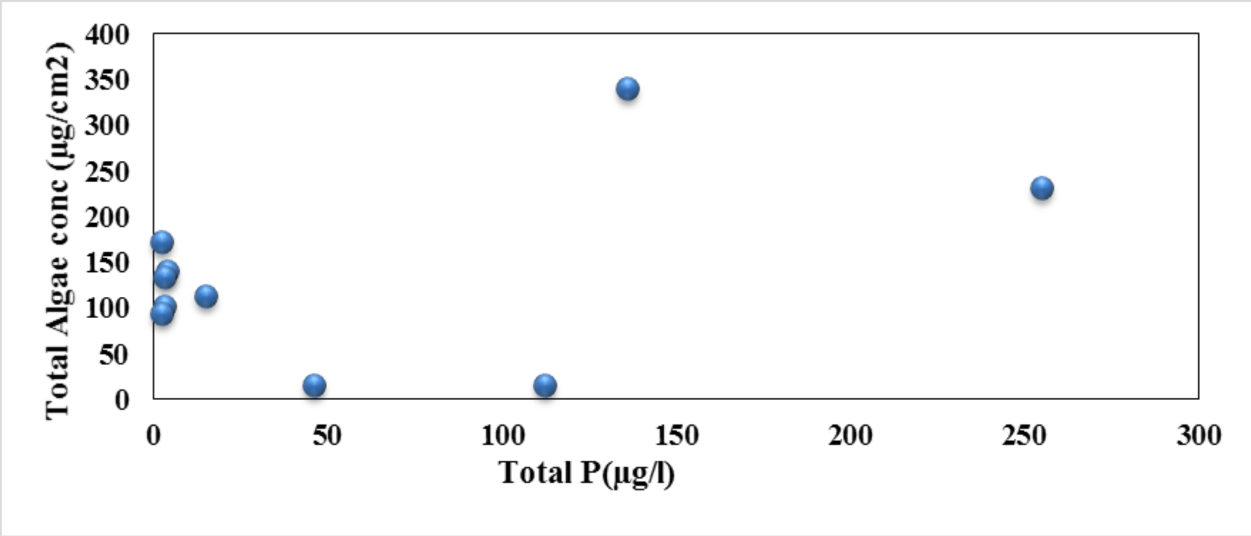

Fig.7: Relationship between algal chlorophyll and stream total phosphate concentration Algal bio-mass was positively associated the streams phosphate levels. (R^2=0, 0597 and P= 0,017).

Abiotic variation along the nutrient gradient:

Several abiotic variables increased along the nutrient gradient. These include other total Phosphorus, other nutrients, such as ammonium, which increased as phosphate increased. Additionally, turbidity, silicate, conductivity, alkalinity and sulphate (Table 3, Fig.8).

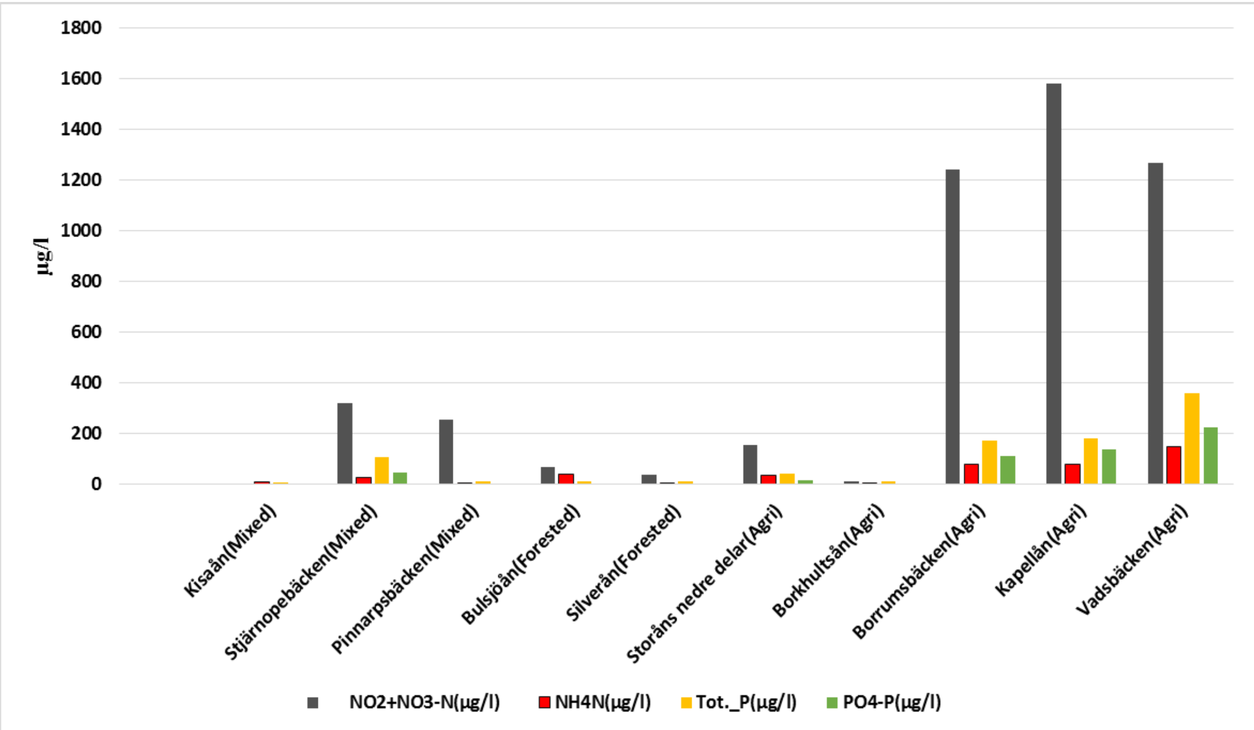

Fig. 8: Nutrient trend on different streams.

Partial least squares regression:

In PLS analysis of decomposition in the coarse mesh bags, the most important predictors in the model were the extent of stream turbidity, with higher levels associated to faster litter decomposition (Table 2). Other important predictors that were also positively associated with litter decomposition were ammonium, phosphate, silicate, nitrates, DOC, and sulphate (Table 2). However, mean insect detritivore abundances was negatively associated with litter decomposition in the stream (Table 2).

Table 2: Variable important predictors obtained from PLS regression analyses of litter decomposition (K-degree day in coarse mesh bags). Only variables with VIP > 0.7 are shown, reflecting the cutoff for inclusion in the model.

	Variable	VIP	Coefficient
Coarse	Turb. FNU	14,882	0,1328
	NH_4_N µg/l	14,016	0,125
	PO_4_P µg/l	12,916	0,1152
	Si mg/l	12,529	0,1118
	NO_2+NO_3_N µg/l	10,806	0,0964
	DOC(mg/l)	0,8219	0,0733
	Mean insect detritivores per litter bag	0,8105	-0,0723
	SO_4_IC mekv/l	0,7475	0,0667

The most significant predictors from PLS analyses of final algal biomass were stream alkalinity, with higher values associated with faster algal biomass accrual (Table 3). Other important predictors that were also positively related to final algal biomass were phosphate, nitrate, and pH. In contrast, DOC, turbidity, silicate and ammonium were negatively associated with litter decomposition in the stream (Table 3).

Table 3: Variable important predictors obtained from PLS regression analyses of stream algal biomass accrual rate only variables with VIP > 0.7 are shown, reflecting the cutoff for inclusion in the model.

Total algae	**Alk./Acid mekv/l**	**13,142**	**0,363**
	pH	11,184	0,3302
	DOC(mg/l)	10,878	-0,3299
	Turb. FNU	10,216	-0,1635
	SO_4_IC mekv/l	0,9782	0,203
	Si mg/l	0,88	-0,0981
	PO_4_P µg/l	0,8569	0,0279
	NH_4_N µg/l	0,8342	-0,0079
	NO_2+NO_3_N µg/l	0,7961	0,1229

Discussion

Evaluation of hypothesis:

My results confirmed hypothesis (i) with the most agricultural sites generally characterized by the higher nutrient concentration and sediment loads (Table. 1 and figure. 8). However, hypothesis (ii) that nutrients would stimulate both leaf decomposition and algal biomass accrual, was only confirmed for decomposition in the coarse mesh bags. Algal biomass accrual did not vary with increasing nutrients, with PLS analyses indicating limitation of growth especially by DOC, which was high in the reference sites, and turbidity, which was highest in the most agricultural sites. Finally, hypothesis (iii) was confirmed, with detritivore abundance declining with increasing nutrients.

Litter decomposition and the effects of different factors (based on PLS):

My results show that litter decomposition increased asymptomatically with increasing phosphorus, though the result was only significant in the coarse mesh bags. This is in line with my prediction for decomposition under hypothesis ii, that increases in nutrients along the gradient stimulate decomposition. The PLS regression indicates that apart from phosphate other nutrients (NO_2+NO_3, NH_4), and silicate and turbidity were all positively associated with decomposition. As nutrients are picked up by the microbes, it stimulates faster litter decomposition, as demonstrated in many previous studies (e.g. Aponte *et al.,* 2012; Couteaux *et al.,* 1995). Robinson & Gessner (2000) also showed nutrient addition can increase feeding activity by invertebrates. They treated some of their leaves with additional N and P, and found that positive effects on microbial activity had knock-on effects on macroinvertebrate feeding. Other studies highlight positive effect of P and NH_4 (Gessner & Chauvet, 1994).

The positive effect of turbidity on litter decomposition could be because an increase in sediment load may lead to litter undergoing physical abrasion (Lepori, Palm & Malmqvist, 2005; Benfield *et al.,* 2001). The positive effect of DOC, and Sulphate in the PLS analysis and some other micronutrients points on a role in litter decomposition, especially in stimulating the activities of heterotrophic microorganisms on leaf litter and hence stepping up the decomposition rates of the litter (Gessner & Chauvet, 1994).

However the PLS model also shows surprisingly that mean detritivore abundances per litter bag was negatively correlated to the litter decomposition. This is contrary to what we expected because detritivores cause fragmentation of leaf litter (Gessner *et al.,* 1999) and they also reduce the particle size of the litter there by facilitating the decomposition process (Hieber & Gessner, 2002). My results might be so because in the most turbid streams where detritivore densities were high, increased sediment load may bury the litter and also the high flow (Table 1) which may prevent the detritivore to easily eat them (e.g. Lepori *et al.,* 2005). Or it could be that although the detritivore species were of high biomass, their taxonomic composition may have changed to species less capable of eating the leaves (i.e. with softer mouthparts). These alternatives could be assessed through experimental studies examining the effects of sediments on detritivore feeding, and through identification of detritivores to species level (Woodward *et al.,* 2012).

As expected, decomposition was more rapid overall in the coarse- than fine-mesh bags, as observed in many previous studies (e.g. McKie & Malmqvist, 2009; Masese *et al.,* 2014), reflecting the stronger effect of invertebrate feeding on mass loss, compared with microbial feeding. The non-significant result in the fine mesh bags might be because the study needed to be run for longer to detect an effect of nutrients on decomposition mediated by microbes alone.

Algal biomass accrual and influences of abiotic factors (Based on PLS):

Agricultural practices typically cause increases in both nutrient and sediment transfer to the streams, as seen in my study. Giller & Malmqvist (1998) showed that when there is increased nutrient runoff to the streams after N and P application in agricultural fields that can enhance growth of algae (Lenat, 1984; Malmqvist & Rundlle, 2002; Karr, 1991; Sharpley *et al.,* 2001). However, in my study algal biomass accrual did not respond to the nutrient gradient as we expected. While some algal biomass accrual was observed in all the studied streams, the relationship between the algal biomass accrual and the land use type was weak to nonexistent. This was contrary to what we expected, given nutrients, and phosphorous in particular, are long recognized as limiting for algal biomass accrual in streams (e.g Dzialowski *et al.,* 2005). The Partial Least Squares regression analysis indicates that turbidity, which was highest in the most agricultural streams, was negatively associated with algal biomass accrual, and this is likely to have contributed to the lack of response by algal biomass accrual to elevated nutrients in the most agricultural streams. Specifically, while nutrients were positively associated with productivity; the negative effect of turbidity appears to have limited the capacity of algal biofilms to respond. This is because an increase in stream turbidity reduces the potential for sunlight to penetrate the water, as needed for normal growth of periphyton (Northcote *et. al.,* 1975; Welch, 1980). The lack of a response in algal biomass accrual may additionally reflect increased scouring of biofilms caused by elevated sediments resulting in abrading of the growing algae. Grazer pressure might also have varied along the gradient (for example increased is agricultural sites and there by limited growth) but I did not quantify this. There was also a negative correlation between the DOC levels and algal biomass accrual in the PLS regression analysis. The DOC effect is likely to reflect attenuation of light, because increased water color reduces light availability (Thrane *et al.,* 2014; Jones, 1992), DOC did not vary systematically along the gradient, but as concentrations were high even in the reference sites, and this could explain limited algal biomass accrual at the low-nutrient end of the gradient. Additionally, studies have shown DOC makes some important elements (for example iron less bioavailable) Thus, there were factors potentially limiting growth at both the low (DOC) and high (turbidity) ends of the gradient.

Comparing litter decomposition and algal biomass accrual:

My litter decomposition assay changed in response to the nutrient gradient, indicating accelerated cycling of leaf litter (organic matter) in response to nutrient enrichment. In contrast, the algal biomass accrual assay might appear not to have given great insights into the effects of agriculture on functioning, given there was no significant result. But in fact my results are revealing: they showed that a key ecosystem process - primary productivity - is potentially suppressed in the more agricultural streams by increased sedimentation and turbidity. My results for both the decomposition and algal biomass accrual assay highlight a major challenge in using functional measures for bio monitoring where to draw “cut off” parameters for determining environmental impairment, or how to judge if functioning in the system is impaired and not able to buffer certain disturbances. In fact, my results highlight the need for other types of environmental assays (both structural and physical) to complement functional assays to draw meaningful conclusions interpretation of a functional assay is difficult without complementary data on the organisms mediating the processes.

Wider perspective:

My results showed that no single assay is sufficient to draw conclusions on the streams health status. There is need for multiple functional assays. If I had studied litter decomposition alone I would have seen a largely expected response to nutrient enrichment, though availability of

additional physico-chemical data allowed a deeper exploration of which factors contributed most to this response. However, the algal biomass accrual assay showed no response to a stronger functional impairment, reflecting the strong effect that high turbidity can have in limiting the response of algal biomass accrual to the increased nutrient values.
The availability of other types of information would increase the insights that could be drawn from my study. For example, information on the diversity of microbes, periphyton and aquatic animals is needed for insights into how biological changes along the gradient affected functioning, while information on stream flow, topographic slope and catchment geology would assist in better understanding fluxes in sediment loads, likely a result of erosion. Such data could also be used to identify mitigation strategies, such as the possibilities for growing vegetation at the catchment site to hold nutrients and sediment, or ridges could be developed to counteract erosion (Hagmann, 1996; Zhou, Shangguan & Zhao 2006; Zhou *et al.,* 2008). From my results it is difficult to be definitive as to whether the nutrient flow or sediment load is a greater problem for overall ecosystem integrity. Nevertheless, it seems clear that reducing turbidity will lead to increased algal biomass accrual at small scales.

Popular science summary

Aquatic ecosystems remain of great value to humans as they are sources of water for drinking and agriculture, while providing recreational services through eg fishing. Aquatic environments are also habitats for other important life forms, such as algae and play a key role in processing and retaining elements like phosphate and nitrogen etc from the atmosphere and the environment. Hence this aquatic environment needs to be fairly stable to maintain these life forms and ecosystem services for example cycling and movement of nutrients, mitigation of floods and droughts and purification of air and water (Boyd & Banzhaf, 2007) but such stability can be undermined by human activities, including excessive inputs of elements like P and N from agricultural landscapes. Therefore it is necessary to monitor stream ecosystems to assess their environmental status, so that important life forms and the chemical features of the water does not change too much to render it undrinkable and less habitable to aquatic life forms.

Traditionally, stream ecologists have used changes in biodiversity as means to measure the stability of aquatic ecosystems (ecosystem status). These methods are good for detecting shifts in freshwater communities but may miss changes in the way ecosystems function, which limits insights about ecosystem services. Hence, today functional bioassays are now used to complement these methods. I used two measures of ecosystem functioning, leaf litter decomposition and algal biomass accrual, to monitor ecosystem status in ten streams in Sweden which represented a gradient of increasing agricultural disturbance, as represented by increasing nutrient concentrations. There is an increased nutrient load for example P and N in the agricultural streams relative to forested streams. The results also show that the agricultural streams have increased sediment loads compared to the mixed and forested streams. Litter decomposition increased asymptotically with increases in nutrient level (for example N and P. In contrast, algal biomass accrual though did not reflect the stream nutrient gradient. My results showed that no single assay is sufficient to draw conclusion of the streams health status. There is need for multiple functional assays. If I had studied litter decomposition alone I would have seen a largely expected response to nutrient enrichment, though availability of additional physico-chemical data allowed a deeper exploration of which factors contributed most to this response. However, the algal biomass accrual assay in showing no response points to a stronger functional impairment, reflecting the strong effect that high turbidity can have in limiting the response of algal biomass accrual to the increased nutrient values.

Acknowledgement

Thanks to my supervisor Brenden Mckie for helping me in the laboratory and supporting me throughout my thesis and thanks to Amélie Truchy for supporting me during lab and field work, thanks to Maria Kahlert and Suchintha Alahakoon for reading my work and special thanks to Ndobe Ernest for helping me to edit the work.

Finally, I thank my family for supporting me throughout all my studies at University.

References

Alexandratos, N., & Bruinsma, J. (2012). World agriculture towards 2030/2050: the 2012 revision. *ESA Working Paper*. 12-03.

Allan, J. D. (2004). Landscapes and riverscapes: the influence of land use on stream ecosystems. *Annual Review of Ecology, Evolution, and Systematics*, 257-284.

Alster, C. J., German, D. P., Lu, Y., & Allison, S. D. (2013). Microbial enzymatic responses to drought and to nitrogen addition in a southern California grassland. *Soil Biology and Biochemistry*, 64, 68-79.

Aponte, C., García, L. V., & Maranon, T. (2012). Tree species effect on litter decomposition and nutrient release in mediterranean oak forests changes over time. *Ecosystems*, 15(7), 1204-1218.

Arheimer, B., & Liden, R. (2000). Nitrogen and phosphorus concentrations from agricultural catchments—influence of spatial and temporal variables. *Journal of Hydrology*, 227(1), 140-159.

Asonye, C. C., Okolie, N. P., Okenwa, E. E., & Iwuanyanwu, U. G. (2007). Some physico-chemical characteristics and heavy metal profiles of Nigerian rivers, streams and waterways. *African Journal of Biotechnology*, 6(5), 617.

Benfield E.F. (1996) Leaf breakdown in stream ecosystems. In: McKie, B. G., & Malmqvist, B. (2009). Assessing ecosystem functioning in streams affected by forest management: increased leaf decomposition occurs without changes to the composition of benthic assemblages. *Freshwater Biology*, *54*(10), 2086-2100.

Benfield, E. F., Webster, J. R., Tank, J. L., & Hutchens, J. J. (2001). Long-Term Patterns in Leaf Breakdown in Streams in Response to Watershed Logging. *International Review of Hydrobiology, 86*(4-5), 467-474.

Benke, A. C. (1990). A Perspective on America's Vanishing Streams. *Journal of the North American Benthological Society, 9*(1), 77-88.

Boon, P. J. (2000). The development of integrated methods for assessing river conservation value .*Springer Netherlands,* 422, 413-428.199.

Boyd, J., & Banzhaf, S. (2007). What are ecosystem services? The need for standardized environmental accounting units. *Ecological Economics*, *63*(2), 616-626.

Bremner, J., López-Carr, D., Suter, L., & Davis, J. (2010). Population, poverty, environment, and climate. *Interdisciplinary Environmental Review, 11*(2/3), 112–126.

Bunn, S. E., & Davies, P. M. (2000). Biological processes in running waters and their implications for the assessment of ecological integrity.*Springer Netherlands*, *422*, 61-70.

Bunn, S. E., Davies, P. M., & Mosisch, T. D. (1999). Ecosystem measures of river health and their response to riparian and catchment degradation. *Freshwater Biology, 41*(2), 333-345.
Canhoto, C., & Graça, M. A. (2006). Leaf litter processing in low order streams. *Limnetica, 25*(1-2), 1-10.

Carpenter, S. R., Kitchell, J. F., & Hodgson, J. R. (1985). Cascading Trophic Interactions and Lake Productivity. *BioScience, 35*(10), 634-639.

Couteaux, M. M., Bottner, P., & Berg, B. (1995). Litter decomposition, climate and liter quality. *Trends in Ecology & Evolution*, 10(2), 63-66.

Cummins, K. W. (1974). Structure and function of stream ecosystems. *BioScience*, *24*(11), 631-641.

Cummins, K. W., & Klug, M. J. (1979). Feeding Ecology of Stream Invertebrates. *Annual Review of Ecology and Systematics, 10*, 147-172.

Cummins, K. W., Petersen, R. C., Howard, F. O., Wuycheck, J. C., & Holt, V. I. (1973). The Utilization of Leaf Litter by Stream Detritivores. *Ecology, 54*(2), 336-345.

Dynesius, M., & Nilsson, C. (Eds.). (1994). Fragmentation and flow regulation of river systems in the northern third of the world. *Science-New York then Washington, 266*, 753-753.

Dzialowski, A. R., Wang, S.H., Lim, N.C., & Spotts, W. W. (2005). Nutrient limitation of phytoplankton growth in central plains reservoirs, USA. *Journal of Plankton Research, 27*(6), 587-597.

Eriksson, L., Johansson, E., Kettaneh-wold, N. and Wold, S. (1999). Introduction to Multi and Megavariate Data Analysis Using Projection Methods (PCA and PLS). U Metrics AB, Umeå, Sweden.

Gannon, J. E., & Stemberger, R. S. (1978). Zooplankton (especially crustaceans and rotifers) as indicators of water quality. *Transactions of the American Microscopical Society, 97*, 16-35.

Gessner, M. O., & Chauvet, E. (1994). Importance of Stream Microfungi in Controlling Breakdown Rates of Leaf Litter. *Ecology, 75*(6), 1807-1817.

Gessner, M. O., & Chauvet, E. (2002). A case for using litter breakdown to assess functional stream integrity. *Ecological Application, 12*(2), 498-510.

Gessner, M. O., Chauvet, E., & Dobson, M. (1999). A perspective on leaf litter breakdown in streams. *Oikos, 85*(2), 377-384.

Gibbs, K. E., Mackey, R. L., & Currie, D. J. (2009). Human land use, agriculture, pesticides and losses of imperiled species. *Diversity and Distributions, 15*(2), 242–253.

Giller, P. S., & Malmqvist, B. (1998). *The Biology of Streams and Rivers.* Oxford University Press.

Godfray, H. C., Beddington, J. R., Crute, I. R., Haddad, L., Lawrence, D., Muir, J. F., . . .Toulmin, C. (2010). Food Security: The Challenge of Feeding 9 Billion People. *Science, 327* (5967), 812-818.
Grantz, D. A., Garner, J. H. B., & Johnson, D. W. (2003). Ecological effects of particulate matter. *Environment International*, 29(2), 213-239.

Hagmann, J. (1996). Mechanical soil conservation with contour ridges: Cure for, or cause of, rill erosion? *Land Degradation & Development*, 7(2), 145-160.

Hawkins, C. P., Norris, R. H., Hogue, J. N., & Feminella, J. W. (2000). Development and evaluation of predictive models for measuring the biological integrity of streams. *Ecological Applications*, *10*(5), 1456-1477.

Hieberi, M., & Gessner, M. O. (2002). Contribution of stream detritivores, fungi and bacteria to leaf breakdown based on biomass estimates. *Ecology, 83*(4), 1026–1038.

Hladyz, S., Åbjörnsson, K., Giller, P. S., & Woodward, G. (2011). Impacts of an aggressive riparian invader on community structure and ecosystem functioning in stream food webs. *Journal of Applied Ecology, 48(2)*, 443–452.

Jarvie, H. P., Neal, C., & Withers, P. J. (2006). Sewage-effluent phosphorus: A greater risk to river eutrophication than agricultural phosphorus? *Science of the Total Environment, 360*(1-3), 246–253.

Jones, R. I. (1992). The influence of humic substances on lacustrine planktonic food chains. In *Dissolved Organic Matter in Lacustrine Ecosystems*, 73-91. Springer Netherlands.

Kahlert, M., & McKie, B. G. (2014). Comparing new and conventional methods to estimate benthic algal biomass and composition in freshwaters. *Environmental Science: Processes & Impacts, 16*(11), 2627-2634.

Karr, J. R. (1991). Biological integrity: a long neglected aspect of water resource management. *Ecological Applications, 1*(1), 66–84.

Kohler, S. L., & Soluk, D. A. (1997). *Effects of Sedimentation on Stream Communities.* Illinois: Prairie Research Institute.

Lamberti, G. A., Gregory, S. V., & Ashkenas, L. R. (1989). Productive capacity of periphyton as a determinant of plant-herbivore interactions in streams. *Ecology, 70*(6), 1840-1856.

Lamberti, G.A. and Resh, V. (1985). Comparability of introduced tiles and natural substrates for sampling lotic bacteria, algae and macro invertebrates. Fresh water biology, 15 (1), 21-30.

Lenat, D. R. (1984). Agriculture and stream water quality: A biological evaluation of erosion control practices. *Environmental Management, 8*(4), 333-343.

Lenat, D. R. (1993). Using mentum deformities of Chironomus larvae to evaluate the effects of toxicity and organic loading in streams. *Journal of the North American Benthological Society*, 265-269.

Lepori, F., Palm, D., & Malmqvist, B. (2005). Effects of stream restoration on ecosystem functioning: detritus retentiveness and decomposition. *Journal of Applied Ecology, 42*(2), 228–238.
Malmqvist, B., & Rundle, S. (2002). Threats to the running water ecosystems of the world. *Environmental Conservation, 29*(2), 134–153.

Masese, F. O., Kitaka, N., Kipkemboi, J., Gettel, G. M., Irvine, K., & McClain, M. E. (2014). Litter processing and shredder distribution as indicators of riparian and catchment influences on ecological health of tropical streams. *Ecological Indicators, 46*, 23–37.

Matthaei, C. D., Piggott, J. J., & Townsend, R. R. (2010). Multiple stressors in agricultural streams: interactions among sediment addition, nutrient enrichment and water abstraction. *Journal of Applied Ecology, 47*(3), 639–649.

McKie, B.G. and Malmqvist, B. (2009). Assessing ecosystem functioning in streams affected by forest management: increased leaf decomposition occurs without change to the decomposition of benthic assemblage. Freshwater Biology, 54, 2086-2100.

Meyer, J. L., McDowell, W. H., Bott, T. L., Elwood, J. W., Ishizaki, C., Melack, J. M., ... & Rublee, P. A. (1988). Elemental dynamics in streams. *Journal of the North American Benthological Society*, 4(4), 410-432.

Moorhead, D. L., Currie, W. S., Rastetter, E. B., Parton, W. J., & Harmon, M. E. (1999). Climate and litter quality controls on decomposition: an analysis of modeling approaches. *Global Biogeochemical Cycles*, *13*(2), 575-589.

Norris, R. H., & Thoms, M. C. (1999). What is river health? *Freshwater Biology*, 41(2), 197-209.

Northcote, T. G., Ennis, G. L., & Anderson, M. H. (1975). Periphytic and planktonic algae of the lower Fraser River in relation to water quality conditions. *Technical Report No.8*, November *1975. 61 P, 22 Fig, 8 Tab, 70 Ref, 4 Append.*

Pace, M. L., Cole, J. J., Carpenter, S. R., & Kitchell, J. F. (1999). Trophic cascades revealed in diverse ecosystems. *Trends in ecology & evolution*, *14*(12), 483-488.

Palmer, M. A., & Febria, C. M. (2012). The Heartbeat of Ecosystems. Science. *Science, 336*(6087), 1393-1394.

Robinson, C. T., & Gessner, M. O. (2000). Nutrient addition accelerates leaf breakdown in an alpine springbrook. *Oecologia, 122*(2), 258-263.

Rosenberg, R., Blomqvist, M., Nilsson, H. C., Cederwall, H., & Dimming, A. (2004). Marine quality assessment by use of benthic species-abundance distributions: a proposed new protocol within the European Union Water Framework Directive. *Marine pollution bulletin*, 49(9), 728-739.

Sabater, S., Gregory, S. V., & Sedell, J. R. (1998). Community dynamics and metabolism of benthic algae colonizing wood and rock substrata in a forest stream. *Journal of Phycology,* 34(4), 561-567.

Sharpley, A. N., & Withers, P. J. (1994). The environmentally-sound management of agricultural phosphorus. *Fertilizer Research,* 39(2), 133-146.

Sharpley, A. N., McDowell, R. W., & Kleinman, P. J. (2001). Phosphorus loss from land to water: integrating agricultural and environmental management. *Plant and Soil*, 237(2), 287-307.

Swift, M. J., Heal, O. W., & Anderson, J. M. (1979). *Decomposition in terrestrial ecosystems* (Vol. 5). University of California Press.

Thrane, J. E., Hessen, D. O., & Andersen, T. (2014). The absorption of light in lakes: Negative impact of dissolved organic carbon on primary productivity. *Ecosystems*, 17(6), 1040-1052.

Tonin, A. M., & Hepp, L. U. (2011). Effects of nitrate enrichment on leaf litter decomposition. *Acta Limnologica Brasiliensia*, 23(1), 86-94.

Vitousek, P. M., Mooney, H. A., Lubchenco, J., & Melillo, J. M. (1997). Human domination of Earth's ecosystems. *Science*, 277(5325), 494-499.

Wallace, J. B., Webster, J. R., & Cuffney, T. F. (1982). Stream detritus dynamics: regulation by invertebrate consumers. *Oecologia*, 53(2), 197-200.

Welch, E. B. (2002). *Ecological Effects of Waste Water: Applied limnology and pollutant effects*. CRC Press.

Winder, M., & Schindler, D. E. (2004). Climate change uncouples trophic interactions in an aquatic ecosystem. *Ecology*, *85*(8), 2100-2106.

Woodcock, T. S., & Huryn, A. D. (2005). Leaf litter processing and invertebrate assemblages along a pollution gradient in a Maine (USA) headwater stream. *Environmental Pollution*, 134(3), 363-375.

Woodward, G., Gessner, M. O., Giller, P. S., Gulis, V., Hladyz, S., Lecerf, A., ... & Chauvet, E. (2012). Continental-scale effects of nutrient pollution on stream ecosystem functioning. *Science*, 336(6087), 1438-1440.

Yentsch, C. S. (1962). Measurement of visible light absorption by particulate matter in the ocean. Limnology and Oceanography. *Limnology and Oceanography*, 7(2), 207-217.

Zhou, P., Luukkanen, O., Tokola, T., & Nieminen, J. (2008). Effect of vegetation cover on soil erosion in a mountainous watershed. *Catena,* 75(3), 319-325.

Zhou, Z. C., Shangguan, Z. P., & Zhao, D. (2006). Modeling vegetation coverage and soil erosion in the Loess Plateau Area of China. *Ecological Modelling,* 198(1), 263-268.

Zweig, L. D., & Rabeni, C. F. (2001). Biomonitoring for deposited sediment using benthic invertebrates: a test on 4 Missouri streams. *Journal of the North American Benthological Society*, *20*(4), 643-657.